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Blocking IL-10 enhances bacillus Calmette-Guérin induced T helper Type 1 immune responses and anti-bladder cancer immunity

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Abbreviations: BCG, bacillus Calmette-Guérin; DTH, delayed-type hypersensitivity; IFN, interferon; IL, interleukin; IL-10R1, IL-10 receptor 1; mAb, monoclonal antibody; Th, T helper type

Proper induction of Th1 immunity is required for effective immunotherapy of bladder cancer with the bacillus Calmette-Guérin (BCG). Interleukin-10 (IL-10) downregulates the Th1 immune response and is associated with BCG therapy failure. We evaluated BCG plus IL-10 blocking antibodies and found that this combination therapy induces enhanced Th1 immune responses and anti-bladder cancer immunity in preclinical animal models.

Bladder cancer is a common malignant disease dominated by a T helper Type (Th) 2 polarized immunopathologic response. Intravesical instillation of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) has been used for the treatment of superficial bladder cancer for over three decades. BCG therapy can shift the Th2 environment toward a Th1 milieu, leading to effective anti-bladder cancer immunity in the majority of patients. BCG therapy typically results in 50–60% effectiveness against small residual tumors and a 70–75% complete response rate for carcinoma in situ. However, BCG therapy is associated with 40–50% disease recurrence and a lack of therapeutic response in some patients. In addition, up to 90% of patients experience various side effects and occasionally even life-threatening complications such as sepsis. Therefore, the current BCG therapy is not optimal with respect to its efficacy and safety.

To improve BCG therapy, efforts have been made to enhance the induction by BCG of Th1 immune responses, since evidence supports the Th1 immune responses to be essential in BCG-mediated bladder cancer destruction.[1] Cytokines with Th1-stimulating properties such as interferon (IFN)α, interleukin (IL)-2 and IL-12 have been investigated in combination with BCG in the treatment of bladder cancer.[2-4] We previously combined BCG with intravesical IFNα and observed this combination therapy to be safe and effective, particularly in patients who failed to respond to BCG monotherapy.[4] Such BCG and cytokine combination therapies provide an opportunity for the use of lower and safer doses of BCG, while preserving or even enhancing BCG efficacy in the treatment of bladder cancer (**Fig. 1**).

Much progress in the use of BCG in the treatment of bladder cancer has been made thanks to preclinical studies in animal models of bladder cancer. Besides BCG combined with Th1-stimulating cytokines, we have investigated BCG in combination with IL-10 blocking agents for treating bladder cancer in animal models. IL-10 is classified as a Th2 cytokine and regulates growth and/or differentiation of various types of cells to control immune responses and tolerance in vivo.[5] It has been documented that IL-10 plays an inhibitory role in both bladder cancer immunosurveillance and BCG therapeutic efficacy,[1,6] although it can promote antitumor responses in certain types of other cancers. The development of a dominant Th1 cytokine profile (e.g., IFNγ, IL-2 and IL-12) has been associated with the therapeutic efficacy of BCG, whereas the presence of high levels of Th2 cytokines (e.g., IL-10) has been linked with BCG therapy failure.[7] A tendency toward higher ratios of IFNγ vs. IL-10 has also been observed for BCG responders.[7,8] To date, all animal studies have supported the dominance of Th2 cytokines observed in human bladder cancer. Previous studies have shown that IFNγ and IL-12 but not IL-10 are required for local tumor surveillance in a mouse model of bladder cancer, and that IL-10 affect the therapeutic outcome of BCG treatment in animal models of bladder cancer.[9] Mice genetically deficient in IL-10 (*Il10*$^{-/-}$) developed a markedly increased local immune response, coinciding with increased therapeutic efficacy, after intravesical BCG treatment.[9] Accordingly, we have observed that *Il10*$^{-/-}$ mice or mice in which IL-10 had been blocked with neutralizing antibodies developed enhanced delayed-type hypersensitivity (DTH) responses, showing increased mononuclear cell infiltration and production of Th1 cytokines (e.g., IFNγ) in the BCG-treated bladders.[7]

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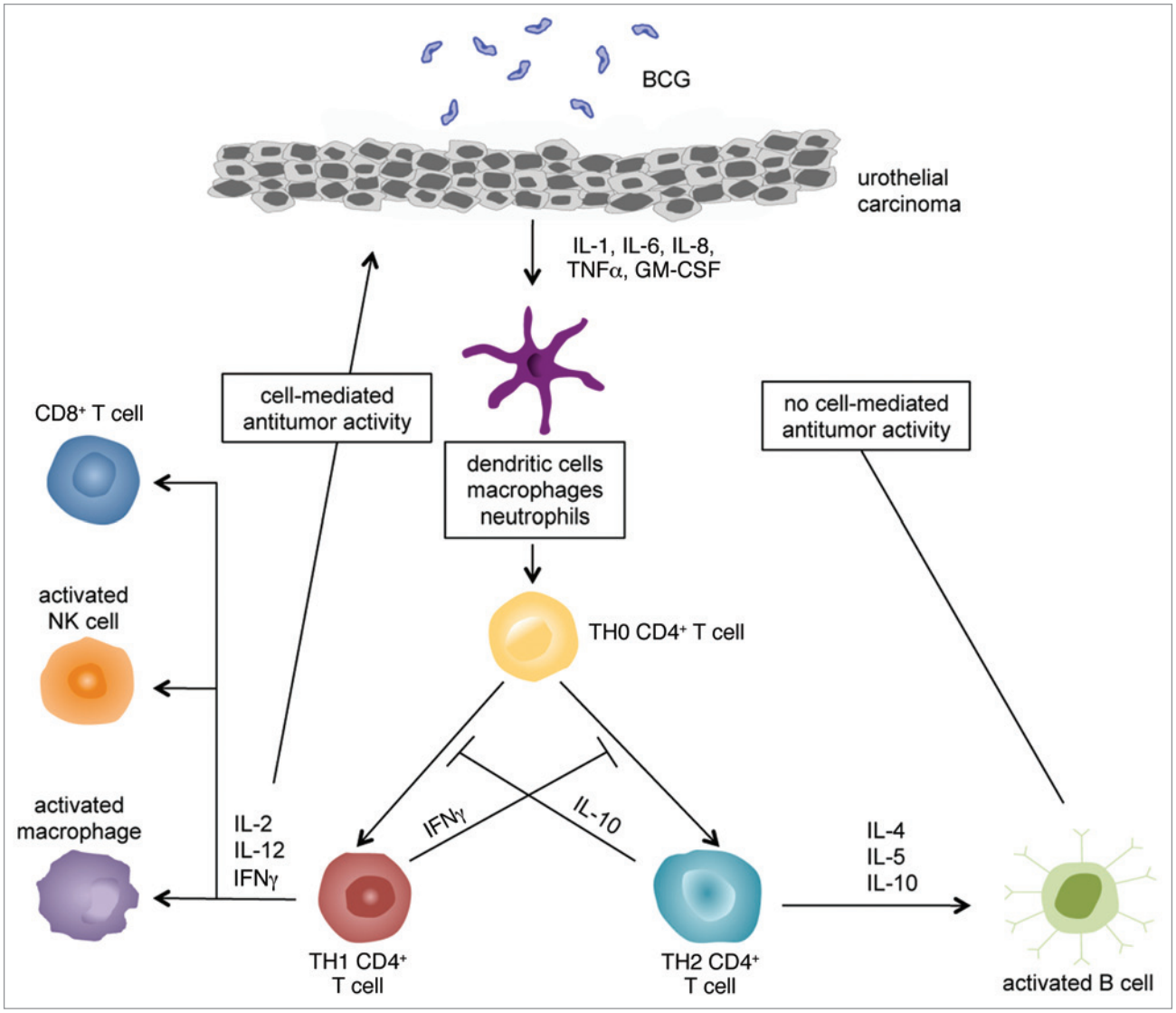

Figure 1. Suggested cascade of immune responses in bladder mucosa induced by intravesical bacillus Calmette-Guérin (BCG) instillation. Attachment of BCG to urothelial cells including carcinoma cells triggers the release of cytokines and chemokines, resulting in the recruitment of various types of immune cells into the bladder wall. Activation of phagocytes and the cytokine environment lead to the differentiation of naïve $CD4^+$ T cells into Th1 and/or Th2 cells, which further direct immune responses toward cellular or humoral immunity, respectively. The therapeutic effect of BCG depends on a proper induction of Th1 immune responses. Interleukin-10 (IL-10) inhibits Th1 immune responses whereas interferonγ (IFNγ) inhibits Th2 immune responses. Thus, blocking IL-10 or inducing IFNγ can lead to a Th1-dominated immunity that is essential for the BCG-mediated bladder cancer resolution.

Under the condition of aggravated DTH responses, a significant enhancement in BCG-induced anti-bladder cancer immunity was also observed,[7] suggesting that blocking IL-10 production and/or activity may provide therapeutic benefits for the BCG-based immunotherapy of bladder cancer.

Recently, we initiated the evaluation of IL-10 blockage at the receptor level on BCG induction of Th1 immune responses and anti-bladder cancer immunity.[10] Mice treated with intravesical BCG plus systemic anti-IL-10 receptor 1 (IL10R1) monoclonal antibodies (mAbs) showed significantly increased IFNγ mRNA and protein in the bladder and urine, respectively, with a dose-dependent pattern. Accordingly, mice implanted with bladder cancer cells and treated with BCG in combination with anti-IL-10R1 mAbs showed substantially improved tumor-free and survival rates compared with control mice. More recently, we further evaluated this approach employing a reduced dose (1/3 full-dose) of BCG plus anti-IL-10R1 mAbs, and observed that the combination therapy significantly prevented bladder cancer metastasis to the lung during an extended experimental period (no metastasis in mice treated with combination therapy vs. 36–53% of incidence in control mice). This observation suggests that BCG might be used at a reduced dose when combined with an IL-10-blocking agent to minimize BCG side effects while maintaining BCG efficacy. The observed effects of anti-IL-10R1 mAbs are presumably due to its inhibition of BCG-induced Th2 immune responses, leading to a Th1 enriched microenvironment in the bladder, a condition essential for the

therapeutic control of bladder cancer by BCG. However, the actual mechanisms by which anti-IL-10R1 mAbs enhances BCG-induced anti-bladder cancer immunity need to be further elucidated. This said, our observations suggest that anti-IL-10R1 mAbs might serve as an effective agent in the treatment of bladder cancer, particularly for high-risk patients, when combined with BCG. A humanized form of anti-IL-10R1 mAbs warrants future investigation for BCG treatment of bladder cancer. As research continues, we anticipate that a new BCG therapy with improved efficacy and limited side effects will be available for bladder cancer, one of the most frequently occurring and most expensive cancers to treat.

References

1. Brandau S, Suttmann H. Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer: a success story with room for improvement. Biomed Pharmacother 2007; 61:299-305; PMID:17604943; http://dx.doi.org/10.1016/j.biopha.2007.05.004.
2. Luo Y, Chen X, Downs TM, DeWolf WC, O'Donnell MA. IFNalpha 2B enhances Th1 cytokine responses in bladder cancer patients receiving *Mycobacterium bovis* bacillus Calmette-Guérin immunotherapy. J Immunol 1999; 162:2399-405; PMID:9973521.
3. O'Donnell MA, Luo Y, Chen X, Szilvasi A, Hunter SE, Clinton SK. Role of IL-12 in the induction and potentiation of IFNγ in response to bacillus Calmette-Guérin. J Immunol 1999; 163:4246-52; PMID:10510362.
4. O'Donnell MA, Boehle A. Treatment options for BCG failures. World J Urol 2006; 24:481-7; PMID:17021823; http://dx.doi.org/10.1007/s00345-006-0112-0.
5. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001; 19:683-765; PMID:11244051; http://dx.doi.org/10.1146/annurev.immunol.19.1.683.
6. Mocellin S, Wang E, Marincola FM. Cytokines and immune response in the tumor microenvironment. J Immunother 2001; 24:392-407; http://dx.doi.org/10.1097/00002371-200109000-00002.
7. Nadler R, Luo Y, Zhao W, Ritchey JK, Austin JC, Cohen MB, et al. Interleukin 10 induced augmentation of delayed-type hypersensitivity (DTH) enhances *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) mediated antitumour activity. Clin Exp Immunol 2003; 131:206-16; PMID:12562379; http://dx.doi.org/10.1046/j.1365-2249.2003.02071.x.
8. Saint F, Patard JJ, Maille P, Soyeux P, Hoznek A, Salomon L, et al. Prognostic value of a T helper 1 urinary cytokine response after intravesical bacillus Calmette-Guerin treatment for superficial bladder cancer. J Urol 2002; 167:364-7; PMID:11743357; http://dx.doi.org/10.1016/S0022-5347(05)65469-9.
9. Riemensberger J, Böhle A, Brandau S. IFNgamma and IL-12 but not IL-10 are required for local tumour surveillance in a syngeneic model of orthotopic bladder cancer. Clin Exp Immunol 2002; 127:20-6; PMID:11882028; http://dx.doi.org/10.1046/j.1365-2249.2002.01734.x.
10. Bockholt NA, Knudson MJ, Henning JR, Maymi JL, Weady P, Smith GJ, III, et al. Anti-IL-10R1 monoclonal antibody enhances BCG-induced TH1 immune responses and antitumor immunity in a mouse orthotopic model of bladder cancer. J Urol 2012; 2228-35; http://dx.doi.org/10.1016/j.juro.2012.01.030.